

## A FIELD EVALUATION IN MEXICO OF FOUR BAITS FOR ORAL RABIES VACCINATION OF DOGS

MARIA G. FRONTINI, DANIEL B. FISHBEIN, JUAN GARZA RAMOS, ESTRELLA FLORES COLLINS, JUAN MANUEL BALDERAS TORRES, GUADALUPE QUIROZ HUERTA, JOSE DE JESUS GAMEZ RODRIGUEZ, ALBINO J. BELOTTO, JAMES G. DOBBINS, SAMUEL B. LINHART, AND GEORGE M. BAER

*Viral and Rickettsial Zoonoses Branch, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia; Productora Nacional de Biologicos Veterinarios, Mexico, D.F., Mexico; Centro de Salud, Jurisdiccion de Atlixco, Puebla, Mexico; Servicios Coordinados de Salud Publica de Puebla, Puebla, Mexico; Field Epidemiology Training Program, Ministry of Health, Mexico, D.F., Mexico; Pan American Health Organization, Zone Office, Mexico, D.F., Mexico; Denver Wildlife Research Center, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Denver, Colorado*

**Abstract.** We evaluated four baits for the delivery of oral rabies vaccines to dogs. In a controlled study in a town in rural Mexico, 177 randomly selected dogs were assigned to receive one of four experiential baits (two of which were developed by the Denver Wildlife Research Center [DWRC]): one of two cylindrical polyurethane sponges with a corn meal coating (one fried in corn oil [DWRC-corn], the other in fish oil [DWRC-fish]), a fish-flavored polymer bait, or a wax bait. Each dog was also offered a commercial dog biscuit. We recorded whether or not the bait was completely consumed, and used the following measures to estimate the amount of oropharyngeal contact with each bait: total chewing time, presence of pieces of bait on the ground following administration, the total area of ground surrounding the location of ingestion that was covered with green dye contained in each bait, and condition of ampules that contained the dye. The dog biscuits were completely consumed significantly more often than the baits (155 of 176 [88%] for the biscuits versus 89 of 176 [50.5%] for the four baits;  $P < 10^{-4}$ ), but were chewed for a significantly shorter time than the baits (mean time 34 sec for the biscuit versus 60-82 sec for the four baits;  $P < 0.001$ ). The ideal bait would probably combine the attractiveness of the commercial biscuit and the ability of the sponge baits to promote contact with the mucous membranes.

Mass vaccination programs in developed countries and in several Latin American countries have been effective in preventing rabies in dogs, resulting in a marked reduction in the incidence of both human rabies and postexposure rabies treatments.<sup>1,2</sup> In the United States, for instance, the application of such programs reduced the number of rabid dogs from approximately 5,000 annually in the early 1940s to fewer than 200 in the 1980s, with human rabies cases decreasing from over 50 per year to almost none in 1990.<sup>3,4</sup> The incidence of disease differs notably in the developing countries of Africa and Asia, where canine and human rabies are still major public health problems, and where rabid dogs are responsible for more than 90% of the 25,000 human rabies deaths reported worldwide each year.<sup>5</sup>

The decrease in canine rabies in the developed areas of the world (e.g., Europe, United States,

and Canada) has revealed the significant underlying problem of wildlife rabies. The fox, for instance, is the principal rabies reservoir in Europe and Canada.<sup>6</sup> Since rabies control in foxes by population reduction (using poison or traps) has failed,<sup>7</sup> attempts to immunize rather than kill foxes have been made since the 1960s and 1970s,<sup>8-10</sup> with successful oral vaccination programs carried out with attenuated strains of rabies virus in Switzerland in 1978 and in Germany in 1984.<sup>11,12</sup> Foxes and other wild animals, such as raccoons, can be also immunized with recombinant oral vaccines.<sup>13,14</sup>

Since canine rabies programs using parenteral vaccination often fail in developing countries because an insufficient number of dogs are vaccinated, dogs are another important potential target for oral vaccination. Oral vaccination of dogs may make vaccination less laborious than it is today, and may expand public acceptance and

cooperation with canine rabies control. Several oral canine vaccines are under development, but as yet, none have been used in the field.

Oral rabies vaccination requires both effective vaccines and baits to hold the vaccine, attract target animals, and facilitate vaccine ingestion and subsequent contact between the vaccine and the oral mucous membranes.<sup>13</sup> Several kinds of baits for foxes and raccoons as well as systems for distributing those baits were developed and evaluated during the 1970s and 1980s.<sup>11-14, 16, 17</sup> In contrast, the use of baits for possible delivery of oral rabies vaccine to dogs has not been well studied.<sup>18</sup> We therefore conducted studies in a rural area of a developing country where canine rabies is endemic. This report describes a field evaluation of four types of dog baits that we studied in central Mexico.

#### MATERIALS AND METHODS

The bait trials were carried out in a small village in Puebla state, Mexico. A house-to-house census of the entire village identified 292 households with a total of 1,824 people and 710 dogs. The census questionnaire included questions about the dogs in each household (i.e., name, age, and sex).

Before the study began, we randomly selected 245 (34.5%) dogs from a complete canine census we conducted for a companion investigation,<sup>19</sup> and randomly assigned one of four bait types to be used in each trial. A commercial dog biscuit was also offered to each test dog (as a control). The address of the dog's owner was obtained from the census, and the house was visited. One team member explained the purpose of the study to the owner, determined if the dog was available, gave the bait (previously randomly assigned for that dog) to the owner, and measured the time for the dog to contact, chew, swallow, and completely ingest the bait. The second person recorded the study data. When households were visited and the owner or dog was not at home, the house was revisited daily for two days in attempts to enroll the dog in the study.

Two of the baits, developed and produced by the Denver Wildlife Research Center (DWRC) of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture, were composed of a cylindrical polyurethane sponge (1.5 cm × 5.5 cm) that had a cavity to hold a wax ampule and had been dipped in a commercial

food batter mix of corn meal, milk, and egg. The baits were then deep fried, one in corn oil (DWRC-corn), and the other in fish oil (DWRC-fish). These baits were similar to those developed for raccoons by Linhart and others,<sup>20</sup> except that these baits were hardened by exposure to air, making it necessary for the dogs to chew rather than gulp them.

The other two baits, a fish-flavored polymer bait and a wax bait, had been developed to deliver oral rabies vaccine to raccoons and foxes, respectively.<sup>21, 22</sup> The wax bait had a 2 × 2 × 1-cm polystyrene blister pack, while the other three baits contained paraffin ampules (5 cm × 0.8 cm) filled with 2 ml of green vegetable dye. The ampule simulated the vaccine container intended for future oral vaccination of dogs. As a control, a medium-sized commercial dog biscuit (The IAMS Co., Dayton, OH) was presented to each dog, and the same information was recorded as with the other baits, except for information about the dye (there was no dye or dye ampule in the biscuits). The order of presentation of the biscuits was varied, with the dogs receiving the biscuit either before or after the bait to determine if initial exposure to one or the other influenced bait acceptance, or the speed or completeness of bait consumption.<sup>23</sup>

After the trial protocol was explained to the dog's owner, each dog was brought to the investigator. When there was more than one dog in a household, the subject dog was isolated as much as possible from the others during the trial. To begin the trial, each owner placed the bait on the ground in front of the animal; then a member of the team recorded the time elapsed to the dog's initial physical contact with the bait, initial chewing, initial swallowing, and complete consumption (no bait remaining on the ground). The trial was terminated when the dog swallowed the bait or after a maximum of 180 sec.

The following measures were used to estimate the amount of oropharyngeal contact (the tissue presumed critical for proper immunization by the oral route): 1) the total chewing time, 2) the presence of pieces of bait found on the ground (indicating partial consumption of the bait), 3) the condition of the paraffin ampule (unbroken, partially chewed, or swallowed), and 4) the total area of the ground covered with green dye (this area was estimated by comparing the area of spillage with the size of various shaded circles on a field data sheet).

TABLE 1  
Percentage of baits chewed, swallowed, and completely ingested\*

Bait type	Trials initiated	Physical contact		Chewing		Swallowing		Complete ingestion	
		no.	(%)	no.	(%)	no.	(%)	no.	(%)
Wax	39	29	(74)	17	(44)	10	(26)	4	(10)
DWRC-corn	51	48	(94)	45	(88)	41	(80)	34	(67)
Polymer	48	46	(96)	43	(90)	35	(73)	24	(50)
DWRC-fish	39	35	(90)	33	(85)	29	(74)	27	(69)
Biscuit	176	176	(100)	171	(97)	169	(96)	155	(88)

\* DWRC = Denver Wildlife Research Center.

The animal's behavior was also recorded as suspicious if the animal appeared aggressive, hesitant, or frightened at the time the bait was presented; otherwise, it was characterized as friendly.

The chi-square test, Fisher's exact test, and Wilcoxon signed rank test were used for statistical comparisons, with  $P < 0.01$  required for statistical significance. The log rank test for homogeneity was used to compare the times for complete bait consumption.<sup>24, 25</sup>

#### RESULTS

Seventy-two percent (177 of 245) of all dogs randomly selected were available for study. Of the 68 dogs that could not be enrolled, 51 were not at home during any of the visits, one was not allowed to participate by the owner, three escaped or had died, and two were too young (< three months). Eleven trials were excluded because the information was incomplete.

The dog biscuits were completely consumed more frequently (88%) than any of the baits ( $P < 0.0001$ ) (Table 1). The most frequently consumed bait was DWRC-fish (69%), followed by DWRC-corn (67%), polymer (50%), and wax (10%). No significant difference was found among the percentages of DWRC-corn, DWRC-fish, or polymer baits consumed, but the proportion of

each of the three baits completely consumed was greater than that of the wax baits. When the order of presentation was changed and the dog biscuit was offered first, the bait acceptance increased slightly, but this difference was not statistically significant.

Results of the four measurements to estimate the amount of oropharyngeal contact were as follows.

1) The total mastication time and the total swallowing times did not differ statistically between bait types, but the dog biscuits were swallowed significantly more rapidly than the baits ( $P < 0.001$ ) (Table 2 and Figure 1).

2) Pieces of bait were left on the ground after bait chewing with 100% of the wax baits, 92% of the polymer baits, 74% of the DWRC-corn baits, 67% of the DWRC-fish baits, and 55% of the biscuits, but the differences were not statistically significant (Table 3).

3) The ampule (or sachet) remained intact (unbroken) following bait consumption in the following percentage of completed trials: 50% with wax baits, 46% with polymer baits, 9% with DWRC-corn baits, and 11% with DWRC-fish baits (Table 3).

4) Dye was noted on the ground in 78% of the trials with the DWRC-fish flavored baits, in 71% of the DWRC-corn bait trials, in 38% of the polymer bait trials, and in none of the four trials in

TABLE 2  
Time between bait presentation and the initiation of chewing, swallowing, and complete ingestion of bait\*

Bait type	Chewing (mean $\pm$ SEM)	Swallowing (mean $\pm$ SEM)	Complete ingestion (mean $\pm$ SEM)
Wax	79 $\pm$ 19.9	61 $\pm$ 18.7	92 $\pm$ 22.9
DWRC-corn	70 $\pm$ 9.5	37 $\pm$ 10.8	92 $\pm$ 10.4
Polymer	82 $\pm$ 6.8	57 $\pm$ 8.5	105 $\pm$ 9.1
DWRC-fish	60 $\pm$ 7.7	36 $\pm$ 7.2	75 $\pm$ 8.2
Biscuit	34 $\pm$ 1.7	22 $\pm$ 1.7	47 $\pm$ 2.2

\* All times are in seconds. DWRC = Denver Wildlife Research Center.

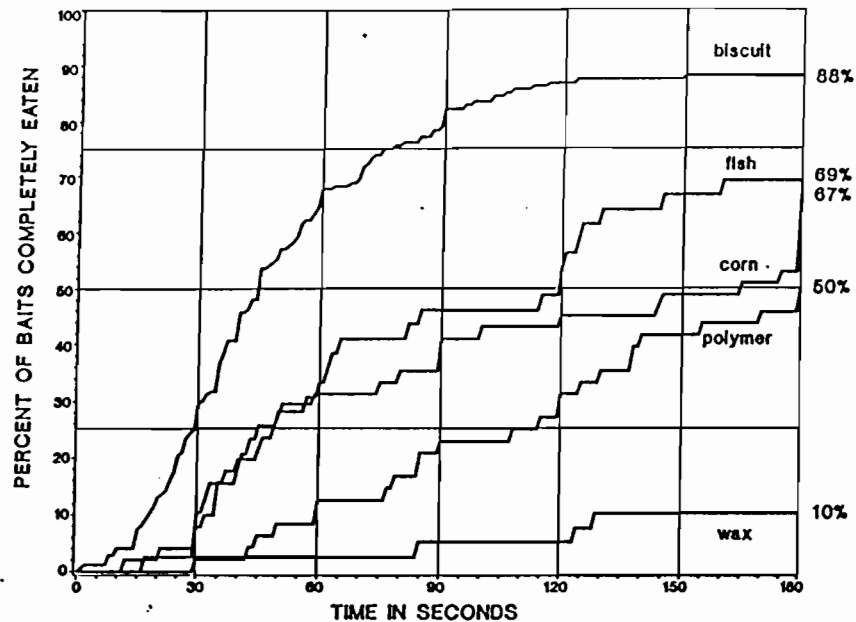


FIGURE 1. Cumulative proportion of baits and biscuit completely consumed, by time since initiation of trial in Atlixco, Mexico, 1990. biscuit = a medium-sized commercial dog biscuit (The IAMS Co., Dayton, OH); fish = a cylindrical polyurethane sponge (1.5 cm × 5.5 cm) that had a cavity to hold a wax ampule and had been dipped in a commercial food batter mix of corn meal, milk, and egg, and deep fried in fish oil; corn = a cylindrical polyurethane sponge (1.5 cm × 5.5 cm) that had a cavity to hold a wax ampule and had been dipped in a commercial food batter mix of corn meal, milk, and egg deep fried in corn oil similar to those developed for raccoons by Linhart and others;<sup>20</sup> polymer = a fish-flavored polymer bait developed to deliver oral rabies vaccine to raccoons;<sup>21</sup> wax = a wax block (2 × 2 × 1 cm) with a polystyrene blister pack in the center.<sup>22</sup>

which dogs completely ingested the wax baits (Table 3). There was no statistical difference between the proportion of trials in which dye was found on the ground with the DWRC-corn and DWRC-fish baits. However, dye was found on the ground significantly more often with the DWRC-corn and DWRC-fish baits than with the wax baits or polymer baits. These differences may have been due to the limited ingestion of the polymer and wax baits.

If one considers only those cases in which dogs accepted baits, the biscuit reached 88% of the dog population, which is significantly more often than the DWRC-fish baits (69%), the polymer bait (50%), and the wax baits (10%) (Table 4). If one considers only those cases in which dogs left no pieces of bait or dye as successful, the DWRC-corn baits reached 12%, the DWRC-fish baits reached 8%, the polymer baits reached 2%, and the wax baits reached none of the dogs. (Since

TABLE 3  
Percentage of bait trials with unbroken ampule, pieces of bait, and dye left on the ground after ingestion (trials with complete bait ingestion only)\*

Bait type	Complete ingestion		Ampule unbroken		Pieces on the ground		Dye on the ground		Dye or pieces on the ground	
	(n)		no.	(%)	no.	(%)	no.	(%)	no.	(%)
Wax	4		2	(50)	4	(100)	0		4	(100)
DWRC-corn	34		3	(9)	25	(74)	24	(71)	28	(82)
Polymer	24		11	(46)	22	(92)	9	(38)	23	(96)
DWRC-fish	27		3	(11)	18	(67)	21	(78)	24	(89)
Biscuit	155		ND		85	(55)	ND		ND	

\* DWRC = Denver Wildlife Research Center; ND = not done (the biscuits did not contain dye or an ampule).

TABLE 4  
Estimators of completeness of placebo vaccine consumption\*

Bait type	Bait completely consumed		Trials without			
	no.	(%)	Pieces on the ground		Pieces or dye on the ground	
			no.	(%)	no.	(%)
Wax	4/39†	(10)	0	(0)	0	(0)
DWRC-corn	34/51	(67)	12/51	(24)	6/51	(12)
Polymer	24/48	(50)	2/48	(4)‡	1/48	(2)
DWRC-fish	27/39	(69)	8/39	(21)	3/39	(8)
Biscuit	155/177	(88)§	23/177	(13)	NA	

\* DWRC = Denver Wildlife Research Center; NA = not applicable.

† Denominator is the total number of trials.

‡  $P < 0.01$  versus other baits, by Fisher's exact test (2-tailed).

§  $P < 0.001$  versus individual baits, by Fisher's exact test (2-tailed).

there was no dye in the biscuits, they could not be included in this comparison.)

To determine why some dogs failed to consume the baits, we studied the relationship between selected epidemiologic and behavioral variables and bait acceptance (Table 5). No statistical differences were found in bait acceptance due to age or sex of the dogs, but dogs with a suspicious attitude were less likely to completely ingest the bait than were dogs with friendly behavior ( $P = 0.002$ ).

#### DISCUSSION

Dogs can be expected to consume those types of food that are more attractive and with which they have had previous experience.<sup>18</sup> Thus, bait acceptance may also vary in different settings, with different dog populations having different food preferences and experiences. The dogs in our study had largely been fed on household leftovers, mainly corn tortillas. This previous experience could have boosted the acceptance of the corn bait, but cannot explain the greater acceptance of the biscuits.

Since no completely safe and effective oral vaccine has been developed for dogs, we cannot determine which of the variables we measured would be most predictive of successful oral vaccination. Oral vaccines for wildlife are believed to be primarily absorbed through the oral mucous membranes and tonsils.<sup>26</sup> With attenuated oral rabies vaccines, there may be substantial (approximately 90%) effective loss of activity when the vaccine is placed in baits.<sup>27</sup> This loss, which may be due to less contact between the vaccine and mucous membranes than when vaccine is placed directly on the tongue, can be com-

pensated for by placing a larger amount or higher concentration of vaccine in the bait. Vaccines may not be immunogenic if vaccine baits are swallowed without chewing since gastric contents are sufficiently acidic to inactivate most attenuated rhabdovirus vaccine strains.<sup>26</sup>

In each bait trial, we considered two factors that we believed would be markers for vaccine acceptance and immunogenicity when baits with vaccines are used: the efficiency of the attraction provided by the odor or taste of the bait matrix (as measured by complete ingestion of the bait), and the efficiency of the bait as a vaccine carrier

TABLE 5  
Bait rejection by age, sex, and behavior of dog\*

Variable	Dogs selected	Trials initiated	Bait rejection
			no. (%)
Age (months)			
0.5-3	24	15	1 (7)
>3-6	16	11	0 (0)
>6-12	15	10	0 (0)
>12-18	39	27	4 (15)
>18-36	63	49	4 (8)
>36	87	64	9 (14)
Total	244	176	18 (10)
Sex			
Male	124	87	8 (9)
Female	110	83	8 (10)
Total	234	170	16 (9)
Behavior			
Suspicious	NA	15	3 (20)†
Friendly	NA	103	3 (3)
Total		118	6 (5)

\* Bait rejection refers to dogs that did not completely ingest either the experimental bait or the biscuit. Totals for variables differ because dogs with missing information were deleted from this analysis. NA = not applicable.

†  $P = 0.002$  versus friendly dogs, by Fisher's exact test (2-tailed).

(as measured by dye on the ground). Physical configuration such as size, color, and texture were characteristics that might have produced a variation in the results. Hardness and friability most likely resulted in the high percentage of trials in which pieces of bait were left on the ground. The ambient temperature during the trials melted the wax baits that were originally for foxes in areas with cold temperatures. The fish-flavored polymer baits were frequently broken into pieces and these pieces were left on the ground; this may have been in part due to their large size. The ampule in these baits appeared as an independent structure that was recognized and kept separate by the dogs. The dogs appeared less able to distinguish between the ampule and bait matrix in both types of DWRC baits (corn and fish); there may have been better contact between the ampule and the bait structure in these two baits.

The commercial dog biscuit was ingested more often than any of the baits. Although no statistical differences were found between the acceptance of the four baits, there was a lower percentage of complete trials in which pieces of DWRC-corn bait were left on the ground, and also a lower percentage of trials in which dye was found on the ground after consumption of this bait. These results suggest that the DWRC-corn bait better fits the requirement of an efficient vaccine carrier. The ampule had been either chewed or swallowed in 91% of the DWRC-corn baits that were completely consumed. However, the commercial dog biscuit appeared to be more attractive to the dogs than any of the baits, suggesting the possibility of combining the attractants in the dog biscuit with the physical configuration of the DWRC baits.

The fact that pieces of the capsule remained on the ground in some cases with all baits indicates the potential for suboptimal vaccine delivery as well as for human contact with rabies vaccine, and emphasizes the need for a vaccine that is not harmful for humans or other animal species. If such a vaccine was available, baits might be distributed to unowned dogs, thus increasing the total vaccine coverage.<sup>18</sup>

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**Authors' addresses:** Maria G. Frontini, Department of Public Health, Veterinary School, National University of La Plata, La Plata, Argentina. Daniel B. Fishbein, Global EIS Program, Centers for Disease Control, Atlanta, GA 30333. Juan Garza Ramos, Centro Interamericano de Estudios de Seguridad Social, Avenida San Ramon S/N y Esquina San Geronimo, Unidad Independencia, Mexico DF, 10100 Mexico. Estrella Flores Collins, Jurisdiccion Sanitaria #5, Atlitico, Puebla, Mexico. Juan Manuel Balderas Torres, Oficina de Control de Zoonosis, Servicios Coordinados de Salud en el Estado de Puebla, Puebla, Mexico. Guadalupe Quiroz Huerta, Direccion Epidemiologia, Secretaria de Salud, FCO de P. Miranda 177, 6° Piso, Col. Merced Gomez, CP 01600, Mexico DF, Mexico. Jose de Jesus Gamez Rodriguez, Direccion Epidemiologia, Secretaria de Salud, FCO de P. Miranda, 177, 1° Piso, Col. Merced Gomez, CP 01600, Mexico DF, Mexico. Albino J. Belotto, Asesor en Salud Publica Veterinaria, Representacion OPS/OMS en Bolivia, Casillas Postales 9790 y 2504, La Paz, Bolivia. James C. Dobbins, Viral Exanthems and Herpes Virus Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333. Samuel B. Linhart, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens GA 30602. George M. Baer, Laboratorios Baer, Mexico DF, Mexico.

Reprint requests: Daniel B. Fishbein, International Branch, DFE, EPO, Centers for Disease Control, 1600 Clifton Road, Mailstop C-08, Atlanta, GA 30333.

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